CHROMOSOME COUNTS IN *MIMULUS* SECT. *ERYTHRANTHE* (SCROPHULARIACEAE). III

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ABSTRACT

Chromosome counts of $n = 8$ were obtained for greenhouse grown plants of seven populations and color morphs of *Mimulus cardinalis* Douglas, four populations of *M. lewisii* Pursh, and two populations of *M. verbenaceus* Greene. These counts include distinctive populations from the extreme southern peripheries of the ranges of *M. cardinalis* (1 population—3 color morphs) and *M. verbenaceus* (2 populations). Populations of these three species, widespread *M. lewisii* Pursh ($n = 8$), and the rare, narrow endemic, *M. rupestris* Greene ($n = 8$), were hybridized experimentally in 10 intraspecific and interspecific combinations. Eight of the resulting F_1 hybrids showed $n = 8$ and regular chromosome pairing, whereas the other two F_1 hybrids also had two or more regular diploid plants ($n = 8$), but in addition, each had one triploid plant with $n = 12 \pm 1$ or 2.

Evolutionary divergence that involves, for example, chromosome number or homology has been postulated to be both important and likely in isolated peripheral populations or narrow endemic species (Mayr 1976). The purpose of our investigation was to test this hypothesis in *Mimulus* sect. *Erythranthe* by 1) determination of the chromosome numbers in populations of *M. cardinalis* Douglas, *M. lewisii* Pursh, and *M. verbenaceus* Greene, particularly of those from the periphery of their species' ranges, and 2) the study of chromosome pairing in F_1 hybrids, particularly in hybrids between more centrally distributed and more peripheral populations or narrow endemic species (Fig. 1). This investigation is part of the authors' long range experimental study of the evolution of species in sect. *Erythranthe* (Vickery 1978, 1984, Wullstein and Vickery in press).

MATERIALS AND METHODS

Six or more plants of each population were grown in the University of Utah greenhouse (Table 1). The plants were obtained either as

MADROÑO, Vol. 33, No. 4, pp. 264–270, 1986

TABLE 1. CHROMOSOME COUNTS IN *Mimulus* SECT. *Erythranthe*. Populations are arranged by species, locality, collector, culture number. Each culture number is followed by the initial of its species to facilitate recognition.

M. cardinalis Douglas. $n = 8$

U.S.A., CA, Yosemite National Park: Chinquapin, 1858 m, 23 Aug 1984, *S. Sutherland s.n., 13,359C*; Upper Yosemite Falls, ca. 1540 m, 23 Aug 1984, *S. Sutherland s.n., 13,360C*; South Gate, 1544 m, 23 Aug 1984, *S. Sutherland s.n., 13,361C*; Wawona campground, ca. 1230 m, 24 Aug 1984, *S. Sutherland s.n., 13,363C*.

Mexico, Baja California del Norte, Cedros Island, Aguaje Vargas, 600 m, 25 Oct 1981, *S. Sutherland s.n.*, red-flowered morph = *13,248C*; orange-flowered morph = *13,249C*; yellow-flowered morph = *13,106C* and *13,250C*.

M. lewisii Pursh. $n = 8$

U.S.A., WA, Umatilla National Forest, ca. 1250 m, Aug 1980, *R. Jorgensen s.n., 13,098L*; CA, Yosemite National Park, Raisin Lake, 2123 m, 25 Aug 1984, *S. Sutherland s.n., 13,357L*; east of Porcupine Flat, 2461 m, 25 Aug 1984, *S. Sutherland s.n., 13,358L*; UT, Little Cottonwood Canyon, Snowbird, Big Emma, ca. 2525 m, 18 Sept 1984, *R. K. Vickery, Jr. 2928, 13,466L*.

M. verbenaceus Greene. $n = 8$

Mexico, Sonora, Baconora, 770 m, 24 Apr 1982, *D. A. Polhemus s.n., 13,255V*; Yecora, 1550 m, 25 Apr 1982, *D. A. Polhemus s.n., 13,256V*.

transplants from the wild, seeds collected in the wild, or seeds harvested from these sources. Each population was assigned a culture number when it was collected by one of us or when it was obtained from other collectors following the practice of Hiesey et al. (1971).

Experimental hybridizations were made among 10 populations of sect. *Erythranthe* (Table 2) with the goal of assessing chromosome homology, as gauged by chromosome pairing, in the interpopulation and interspecific F_1 hybrids obtained. The populations hybridized included three newly counted ones (Table 1) and seven from an earlier study (Vickery 1978) representative of *M. cardinalis, M. lewisii,* and *M. rupestris* Greene. The crosses were carried out in an insect-free greenhouse. At first, flowers were emasculated, but in later crosses, this practice was discontinued in order to allow some self-pollination. Capsules that contained at least a few seeds resulting from self-pollination developed, whereas capsules with apparently only hybrid seeds often aborted. Also, after the seeds were sown and the seedlings emerged and grew, the "selfed," maternal type seedlings provided an excellent control against which to verify the validity of the F_1 hybrids. Each hybrid combination was assigned a culture number. In experimental studies such as this, culture numbers provide an efficient way of keeping track not only of parental populations (see above), but also of the numerous F_1, F_2 and backcross hybrids studied.

For the chromosome pairing study, six or more plants of each F_1 hybrid combination were propagated in the greenhouse.

TABLE 2. INTERPOPULATION AND INTERSPECIFIC F_1 HYBRIDS OF *Mimulus* ARRANGED BY F_1 HYBRID CULTURE NUMBER, FEMALE PARENT, MALE PARENT, AND UT VOUCHER NUMBER. All parents have $n = 8$ chromosomes. Geographical locations are indicated briefly. For full details of parental populations not listed in Table 1, see Vickery et al. 1958, 1963, 1982; McArthur et al. 1971.

Female parent	Male parent	F_1 culture number
A. *Diploids*, $n = 8$ (all have regular chromosome pairing in Metaphase I of meiosis followed by occasional, slightly irregular segregations).		
M. cardinalis, 7120C Los Trancos, CA	*M. cardinalis, 7113C* Mt. San Antonio, CA	*13,178*
M. cardinalis, 13248C-red Cedros Is., B.C., Mex.	*M. lewisii, 6103L* Ice Lake, CA	*13,293*
M. cardinalis, 13106C-yellow Cedros Is., B.C., Mex.	*M. lewisii, 6103L* Ice Lake, CA	*13,258*
M. verbenaceus, 5924V Grand Canyon, AZ	*M. lewisii, 6103L* Ice Lake, CA	*13,180*
M. lewisii, 5875L Alta, UT	*M. lewisii, 6103L* Ice Lake, CA	*13,374*
M. lewisii, 6103L Ice Lake, CA	*M. lewisii, 5875L* Alta, UT	*13,375*
M. lewisii, 5875L Alta, UT	*M. cardinalis, 13248C*-red Cedros Is., B.C., Mex.	*13,289*
M. lewisii, 13257L Raisin Lake, Yosemite Nat. Park, CA	*M. cardinalis, 13363C* Wawona, Yosemite Nat. Park, CA	*13,468*
M. lewisii, 5875L Alta, UT	*M. rupestris, 9102R* Tepoztlan, Morelos, Mex.	*13,288*
M. rupestris, 9102R Tepoztlan, Morelos, Mex.	*M. cardinalis, 7113C* Mt. San Antonio, CA	*13,179*
B. *Triploids*, $n = 12 \pm 1$ or 2 (each plant exhibited $\pm$ 8 bivalent chromosome associations plus 1–6 univalent chromosomes).		
M. rupestris, 9102R Tepoztlan, Morelos, Mex.	*M. cardinalis, 7113C* Mt. San Antonio, CA	*13,179*
M. lewisii, 5875L Alta, UT	*M. cardinalis, 13248C*-red Cedros Is., B.C., Mex.	*13,289*

Flower buds were removed and the anthers squashed in a drop of aceto-carmine stain. The slides were immediately observed under a phase contrast microscope, an improvement over our earlier method (Vickery et al. 1963). Cover slips were ringed with dental sticky wax, which avoided dehydration and permitted observation for two to three weeks. Usually the chromosomes had taken up sufficient stain to be clearly visible after one or two days. Sketches and camera lucida drawings were made of representative cells. Chromosome number determinations and pairing behavior were based on observations of 20 or more PMC's typically from two to six plants. Vouch-

ers of the plants counted are deposited in the Herbarium of University of Utah (UT).

Results and Discussion

All the native populations counted have $n = 8$ (Table 1). This result confirms previous reports (Brozek 1932, Sugiura 1940, Vickery et al. 1958, 1963, 1982, Hiesey et al. 1971, McArthur et al. 1971) of a uniform $n = 8$ chromosome number in sect. *Erythranthe.* The native, parental populations counted include three from the extreme southern peripheries of the ranges of two species: the populations of *M. verbenaceus* Greene from Baconora and Yecora, Sonora, Mexico and the population of *M. cardinalis* from Cedros Island, Baja California del Norte, Mexico (Table 1). In all cases, the populations had normal meiosis.

The plants of the peripheral populations of *M. verbenaceus* have much narrower leaves compared with plants farther north, particularly those from populations in Arizona that include a topotype from the Verde Valley (Vickery 1978). The narrow leaves of plants from the Baconora and Yecora populations appear intermediate between the lanceolate leaves of typical *M. verbenaceus* and the extremely narrow leaves of *M. nelsonii* Grant (see illustration in Vickery et al. 1963) from Durango and Sinaloa farther south in the Sierra Madre. Plants of *M. cardinalis* populations from Cedros Island are unusual in that individuals have orange, bright yellow, or normal cardinal-red flowers. The three wild color morphs proved to be interfertile as Brožek (1932) had found previously for similar, horticulturally-derived color forms (obtained from Vilmorin et Cie, Paris).

Most of the intra- and interspecific F_1 hybrids (Table 2) exhibited regular chromosome pairing (8 bivalents) in Metaphase I, with occasional irregularities in chromosome segregation in the later stages of meiosis (Fig. 2). Occasional cells with $n = 6$, 7, 9 or 10 chromosomes instead of $n = 8$ were observed. For example, the chromosome counts for one F_1 hybrid (culture no. *13,258*, *M. lewisii* × *M. cardinalis*) were $n = 6$, 1 cell; $n = 7$, 6 cells; $n = 8$, 30 cells; $n = 9$, 2 cells; and $n = 10$, 1 cell.

One plant of each of two of the interspecific F_1 hybrids [culture no. *13,179* = *M. rupestris* (*9102R*) × *M. cardinalis* (*7113C*); and culture no. *13,289* = *M. lewisii* (*5875L*) × *M. cardinalis* (*13,248C*)] were found to be triploids with $n = 12 \pm 2$ chromosomes. The other plants of both F_1 hybrid combinations had $n = 8$ chromosomes and exhibited essentially regular pairing as above. The only other *Erythranthe* polyploid known to us is a single tetraploid F_1 hybrid [*M. nelsonii* (El Salto, Durango, Mexico) × *M. lewisii* (Timberline, Mono Co., CA)] reported by Hiesey et al. (1971). These authors suggest

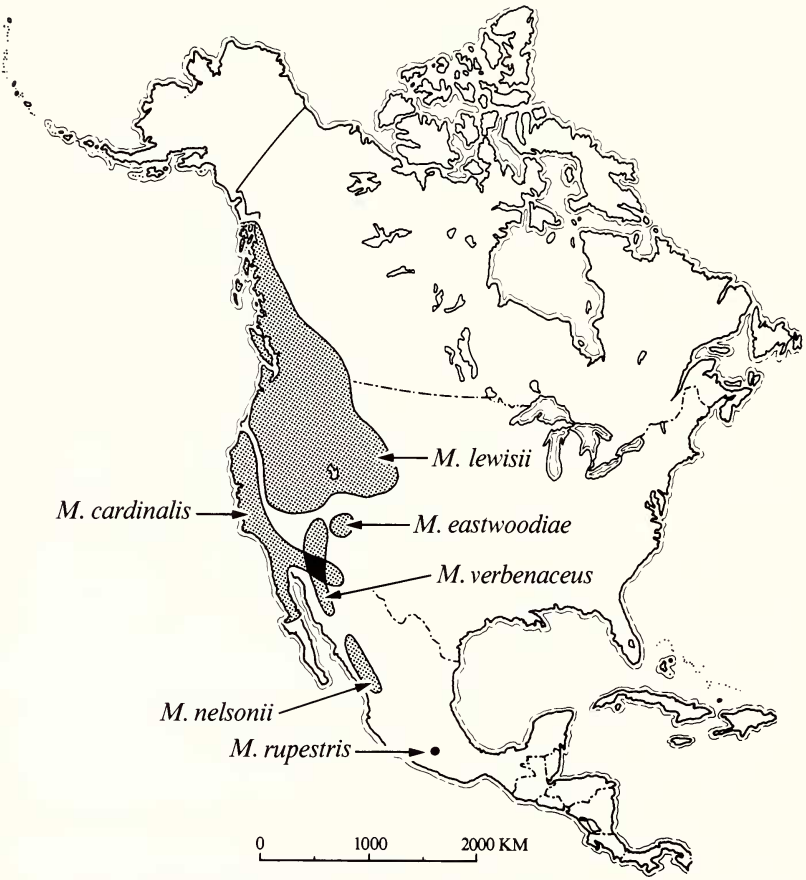

FIG. 1. Distribution map of the species of *Mimulus* sect. *Erythranthe.*

that the formation of this amphiploid tetraploid stresses the genetic distinctness of the *M. cardinalis–lewisii* complex from the *M. eastwoodiae* Rydberg–*verbenaceus–nelsonii* complex to which we have shown *M. rupestris* to belong also (Vickery 1978). Our finding of the triploid F_1 hybrids both within and between these complexes sheds no further light on the relationships of the complexes.

CONCLUSIONS

The peripheral populations of *M. cardinalis* and *M. verbenaceus* have $n = 8$ in accord with other wild populations of sect. *Erythranthe.* No reduction in chromosome pairing or polyploidy was detected in peripheral populations of sect. *Erythranthe* as were observed in sect. *Simiolus,* particularly in *M. guttatus* Fisch. ex DC. (Vickery 1978).

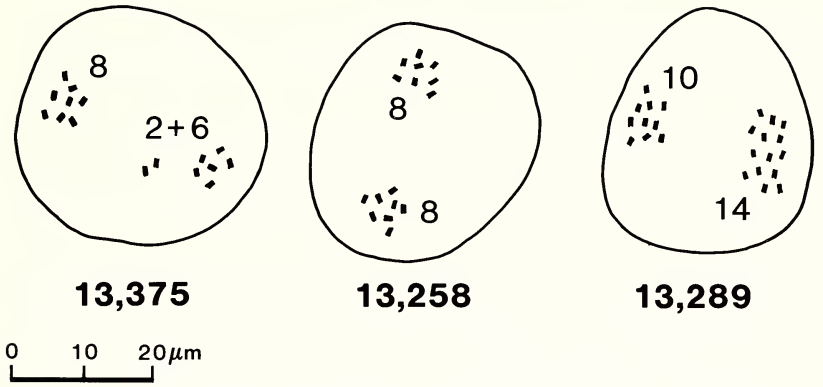

FIG. 2. Camera lucida drawings of representative chromosome configurations at Metaphase II of meiosis. Cultures *13,375* and *13,258* illustrate, respectively, irregular and regular segregations in diploid, $2n = 16$, F_1 hybrids. Culture *13,289* illustrates a typical, irregular segregation in a triploid, $3n = 24$, F_1 hybrid.

The intra- and interspecific F_1 hybrids between populations from the central regions of their species' ranges and peripheral populations showed a similar level of near normal chromosome pairing as did F_1 hybrids both of whose parents came from the central regions of their species' ranges. The F_1 hybrids involving the narrow endemic, *M. rupestris,* also showed normal chromosome pairing. There was no indication that the chromosomes of either the peripheral or narrow endemic populations have evolved to the point where a reduction in homology causes a significant reduction in chromosome pairing.

Our finding of the two rare triploid F_1 hybrid plants suggests to us that polyploidy probably occurs at a very low frequency in any sect. *Erythranthe* population or hybrid as observed in the *M. glabratus* HBK complex (Tai and Vickery 1970). Thus, although the peripheral and narrow endemic populations have diverged morphologically, they do not appear to have diverged in chromosome number or homology in sect. *Erythranthe.*

ACKNOWLEDGMENTS

We thank Drs. Stephen Sutherland, Dan A. Polhemus, and Richard Jorgensen for their helpful collections. We thank N.I.H. (grant no. 507-RR07092) and N.S.F. (grant no. DEB79-11554) for financial support.

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(Received 23 Oct 1985; revision accepted 6 June 1986.)